Remarks

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 26, 28, 33, 117-125, and 127-148 are pending in the application, with 26 being the independent claim. Claims 39 and 40 are sought to be cancelled without prejudice to or disclaimer of the subject matter therein. These changes are believed to introduce no new matter, and their entry is respectfully requested. Support for these amendments can be found at least in the cancelled claims and throughout the specification. Based on the above amendment and the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

Restriction/Election of the Claims

The Examiner has requested that Applicants affirm the election, with traverse, to prosecute Group I, which is drawn to AMV reverse transcriptase of claims 26, 28, 33, 40, 117-125, and 127-148 (Office Action, page 2, last paragraph).

Applicants respectfully disagree with the Examiner's contention that claims 26, 28, 33, 39, 40, 117-125, and 127-148, were unrelated as drawn to ASLV reverse transcriptases. However, in the interest of expediting the prosecution of the present application, Applicants hereby affirm the election to prosecute Group I with traverse and request examination of the claims as directed to AMV.

The Sequence Listing

In the Office Action at page 3, Examiner indicated that the present application is

not in compliance with the sequence rules. Submitted herewith is a disc and paper copy of a substitute sequence listing in compliance with the sequence rules. No new matter has been added by way of the substitute sequence listing. The sequence listing on paper and in computer form are the same.

The Examiner alleges that the application discloses several reverse transcriptases from several viruses and methods of making the viruses and mutants, but no nucleic acid or amino acid sequences are disclosed. According to the Examiner, the specification references specific amino acid residues from an amino acid sequence without identifying the amino acid sequence with a sequence identification number, for example on page 57, lines 11 and 12 of the specification. Applicants respectfully disagree.

According to 37 C.F.R. § 1.821(a), "[s]equences with fewer than four specifically defined nucleotides or amino acids are specifically excluded from this section." Therefore, where Applicants have disclose amino acid sequences with four or more defined nucleotide or amino acids, Applicants have provided a sequence listing thereto and identified said sequences by a sequence identification number. Therefore, Applicants submit that the specification is in compliance with the sequence rules.

The Abstract

The Examiner has suggested that the abstract be limited to 150 words since the space provided for the abstract used by the printer is limited. Applicants have submitted a revised abstract, attached hereto, to accommodate this request.

The Title

The Examiner has alleged that the title is not descriptive. Applicants have

amended the title to "Recombinant Methods for Making Reverse Transcriptases and Mutants Thereof" to accommodate this request.

Objection to the Claims

In the Office Action at page 4, the Examiner has objected to claims 26, 28, 33, 117-125, and 127-148 because they allegedly contain non-elected subject matter.

Applicants have amended the claims to limit them to AMV reverse transcriptases. Therefore, this objection is rendered moot. Withdrawal of this objection is respectfully requested.

The Examiner has objected to claims 26, 28, 33, 117-125, and 127-148 under 37 C.F.R. § 1.75(d)(1) because the claims allegedly state an improper Markush group. Specifically, the Examiner alleges that various members of the Markush group in the claims are different chemical compounds and do not share a common structural feature required for the stated utility of reverse transcriptase activity. Applicants respectfully disagree.

The Markush groups in amended claims 28 and 148 are proper as they relate to subunits of AMV RTs which have reverse transcriptase activity. Therefore, this objection is in error. Withdrawal of the objection is respectfully requested.

The Examiner has also objected to claims 33, 40, and 127-147 under 37 C.F.R. § 1.75(d)(1) for failing to further limit the subject matter of a previous claim. Specifically, the Examiner alleges claims 33 and 40 are dependent on claim 26 and since ASLV reverse transcriptase is assumed to mean AMV reverse transcriptase, the claims do not further limit claim 26. The Examiner further indicated that "units per milligram" is assumed to mean any reverse transcriptase activity. Office Action, page 4, lines 15-19.

Applicants respectfully disagree.

Claim 40 has been cancelled thus rendering this objection moot. Applicants have also amended the remaining claims to AMV reverse transcriptases. In addition, Applicants have amended the claims to further clarify that "units per milligram" relates to units per milligram of polymerase activity. Therefore, reconsideration and withdrawal of this objection are respectfully requested.

The Rejection under 35 U.S.C. § 112, First Paragraph Is Traversed

In the Office Action at page 4, the Examiner has rejected claims 26, 28, 33, 117-125, and 127-148 under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors had possession of the claimed invention. Applicants respectfully traverse this rejection.

Specifically, the Examiner contends that no structure for the nucleic acid/or the amino acid sequences for AMV reverse transcriptase and mutants or the source of the genomic RNA are disclosed. The Examiner further contends that the specification fails to define a unit of activity, teach a preparation of any AMV reverse transcriptase having any kind of activity, or how to make or attribute a transcriptase activity from heterologous dimers of α and/or β subunits from different species of ASLVs (Office Action, page 4, line 33 to page 5, line 6). Applicants respectfully but emphatically disagree.

AMV reverse transcriptases comprise α and/or β subunits (specification at page 3, line 27 to page 4, line 12). The specification clearly discloses how to generate homodimeric, or heterodimeric, or monomeric subunits and their mutants as claimed

with the defined specific activities (specification at page 22, lines 11-16, and especially pages 69-76, 102, and 106). A deposit of *E. coli* DH10B(pDMAVABH-) containing a plasmid coding for the AMV RT α gene (RNase H^+) and AMV RT β gene (RNase H^-) was deposited as disclosed on page 75, lines 10-13. Using the deposited plasmid, one of ordinary skill in the art can easily produce homodimeric, heterodimeric, monomeric, or mutant subunits of AMV RTs using routine experimentation.

Moreover, methods of measuring a polymerase unit of activity are already well known in the art. In addition, the specification also describe methods for determining polymerase unit activity at pages 63 and 99. Therefore, this rejection is in error; withdrawal of the rejection is respectfully requested.

The Rejections under 35 U.S.C. § 112, Second Paragraph Are Traversed

In the Office Action at page 5, the Examiner has rejected claims 26, 28, 33, 40, 117-125, and 127-148 under 35 U.S.C. § 112, second paragraph as allegedly indefinite. Applicants respectfully traverse this rejection.

Specifically, the Examiner contends that abbreviations "ASLV" and "AMV" should be defined once in the claims. Applicants have amended the claim 26, which is the sole independent claim to include the definition of "AMV" to accommodate this portion of the rejection. Therefore, withdrawal of this portion of the rejection is respectfully requested.

The Examiner contends that claim 26 is incomplete for omitting essential steps, specifically a purification step of the reverse transcriptase from the host cell after step (b) and before step (c). Applicants have amended claim 26 to further clarify that which is being claimed thereby accommodating this portion of the rejection. Therefore,

withdrawal of this portion of the rejection is respectfully requested.

Claims 26, 28, 33, 40, 117-125 and 127-148 are allegedly indefinite because of the term "ASLV reverse transcriptase" in claims 26, 28, 33, 40, 117-125, 127-148, "specific activity . . . units per milligram" in claims 26 and 127-148, and "one or more subunits" in claims 28 and 117-120. According to the Examiner, these claims do not clearly set forth the metes and bounds of the patent protection desired. Applicants respectfully disagree.

With regard to "ASLV reverse transcriptase," this term has been cancelled from the claims thus rendering this basis for rejection moot.

With regard to "specific activity . . . units per milligram," Applicants have amended claims 26 and 127-147 to recite "polymerase specific activity" further clarifying that which is being claimed. *See* page 99, lines 10-18 of the specification. Therefore, the claims comport with the requirements under 35 U.S.C. §112, second paragraph. Withdrawal of this portion of the rejection is respectfully requested.

The Examiner further alleges that "one or more subunits" is indefinite because the only enzymatically active form of the ASLV RT enzyme is a dimer and that there could not be more than two subunits per molecule of enzyme. Applicants respectfully but emphatically disagree.

The specification provides evidence that each subunit of ASLV has reverse transcriptase activity, *i.e.* DNA polymerase and RNase H activities and that the catalytically active structurally form can be an α monomer (specification at page 4, lines 10-15, and in Table 8 at page 106). Therefore, the Examiner's assertion that the only enzymatically active form of the ASLV RT enzyme is a dimer is in error. However, Applicants have amended the claims to delete the phrase "one or more subunits" and to

further clarify that which is being claimed. Therefore, Applicants respectfully request that this portion of the rejection be withdrawn.

In the Office Action at page 6, the Examiner alleges that claim 26 is indefinite because of the phrase "having RNase H activity." Applicants traverse this rejection.

The Examiner states that the phrase, in light of the specification, has more than one meaning. According to the Examiner, a first meaning is wild-type RNase H activity and a second could mean having reduced RNase H activity relative to the wild-type. Applicants respectfully submit that the phrase is clear on its face, *i.e.* "having RNase H activity," means having measurable RNase H activity whether it is wild-type or not. However, in the interest of expediting the prosecution of the present application, Applicants have amended claim 26 to delete the phrase "having RNase H activity" to further clarify that which is being claimed. Withdrawal of this portion of the rejection is respectfully requested.

The Examiner has rejected claims 28, 117, 121, and 148 as allegedly indefinite because of the phrase "one or more ASLV reverse transcriptase" in the claims. Applicants respectfully traverse this rejection.

In the interest of expediting the prosecution of the present application, Applicants have amended the claims to limit them to AMV, thus rendering the basis for this portion of the rejection moot. Reconsideration and withdrawal of this portion of the rejection is respectfully requested.

The Examiner has rejected claims 28, 120, and 148 as allegedly indefinite because "βp4 subunit" is not structurally defined by the specification or the claims. Applicants respectfully traverse this rejection.

The βp4 subunit is an ASLV RT subunit which may be processed to produce the

mature β subunit. *See* page 4, lines 23-26. At page 56, the specification teaches that the mature β subunit construct is generated by insertion of a translational stop site at the "p4" subunit cleavage site. Therefore, the Examiner's contention is in error. Withdrawal of this portion of the rejection is respectfully requested.

The Rejection under 35 U.S.C. § 102(b) Is Traversed

In the Office Action at page 6, the Examiner has rejected claims 26, 28, 33, 40, 117-119, 121-125, and 127-148 under 35 U.S.C. § 102(b) as being anticipated by Soltis *et al.* (*Proc. Natl. Acad. Sci. USA 85*:3372-76 (1988)). Applicants respectfully traverse this rejection.

The Examiner contends that "[a]lthough applicant had previously argued similar rejection on the record successfully, this rejection is reinstated because the unit and type of activity is not defined in the specification and/or the claims" (Office Action page 6, lines 4-7). Applicants respectfully disagree.

Claim 26, which claims 28, 33, 40, 117-119, 121-125, and 127-148 depend therefrom, is drawn to a method of producing an AMV reverse transcriptase with a polymerase specific activity of at least 30,000 units per milligram.

Soltis *et al.* do not disclose a method of producing an AMV reverse transcriptase having a polymerase specific activity of at least 30,000 units/milligram. Therefore, the claims clearly are not anticipated by Soltis *et al.* Therefore, this rejection is rendered moot. Withdrawal of the rejection is respectfully requested.

The Rejection under 35 U.S.C. § 103(a) Is Traversed

In the Office Action at pages 7-8, the Examiner has rejected claims 26, 28, 33, 40, 117-119, 121-125, and 127-148 under 35 U.S.C. § 103(a) as being unpatentable over Soltis *et al.* in view of the state of the art at the time the application was filed. Applicants respectfully traverse this rejection.

Specifically, the Examiner contends that "AMV-reverse transcriptase has been used extensively in biotechnology in the preparation of cDNA libraries. Thus, one of ordinary skill in the art would have had motivation at the time of invention to produce AMV-reverse transcriptase by a recombinant method" (Office Action at page 8, lines 1-4). The Examiner further adds that:

> the [person of] ordinary skill in the art would have constructed the pRC23-p95 and pRC23-p63 taught by Soltis and coexpress them in a single host cell to produce the heterodimer . . . one of ordinary skill in the art would be further motivated to construct a single vector comprising the coding sequences for both the α- and β-subunits of AMV-reverse transcriptase under the control of the same promoter which would lead to the production of equal amount of the two subunits (claim 124).

Office Action at page 8, lines 12-18. Applicants respectfully disagree.

Claim 40 has been cancelled thus rendering moot this portion of the rejection.

Claim 26, of which claims 28, 33, 117-119, 121-125, and 127-148 depend therefrom, is drawn to a method of producing an AMV reverse transcriptase with a polymerase specific activity of 30,000 units per milligram.

Soltis *et al.* do not suggest or contemplate a method of producing an AMV reverse transcriptase having a polymerase specific activity of at least 30,000 units/milligram. The Examiner's contention that the deficiencies of Soltis *et al.* are

cured by knowledge available to one of ordinary skill in the art is in error, since this contention has not been supported with any objective evidence or sound scientific reasoning. "Rarely . . . will the skill in the art component operate to supply missing knowledge or prior art to reach an obviousness judgment . . . Skill in the art does not act as a bridge over gaps in substantive presentation of an obviousness case . . . . " *Al-Site Corpn. v. VSI International, Inc.*, 174 F.3d 1308, 1324 (Fed. Cir. 1999). Moreover, an obviousness conclusion must be based on *facts*, rather than on generalities (such as a general and/or unsupported statement of knowledge available in the art). *See In re Warner*, 379 F.2d 1011, 1017 (C.C.P.A. 1967), *cert. denied*, 389 U.S. 1057 (1968); *see also In re Freed*, 425 F.2d 785, 788 (C.C.P.A. 1970). The Examiner has not provided any factual evidence that the skilled artisan would have been motivated to derive the claimed invention with a reasonable expectation of success based on the disclosure of Soltis *et al.* and the general knowledge in the art at the time. Thus, the Examiner has not met the burden of establishing a *prima facie* case of obviousness. Withdrawal of the rejection is respectfully requested.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for

allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,
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Version with markings to show changes made

Claims 39 and 40 have been cancelled.

The following claims have been amended:

26. (Four times amended) A method of producing an Avian Myeloblastosis Virus (AMV) [ASLV] reverse transcriptase having a polymerase specific activity of at least about 30,000 units per milligram [and having RNase H activity], said method comprising

(a) obtaining a host cell comprising one or more nucleic acid sequences encoding [at least one ASLV] AMV reverse transcriptase; and

(b) culturing said host cell under conditions sufficient to produce said [ASLV] AMV reverse transcriptase; and

(c) isolating or purifying said reverse transcriptase thereby obtaining an [ASLV] AMV reverse transcriptase having a polymerase specific activity of at least about 30,000 units per milligram [and having RNase H activity].

28. (Twice amended) The method of claim 26, wherein said [ASLV] AMV reverse transcriptase comprises [one or more subunits] at least one subunit selected from the group consisting of one or more α subunits, one or more β subunits, and one or more βp4 subunits, of [one or more ASLV] AMV reverse transcriptase[s], and fragments or mutants thereof having reverse transcriptase activity.

33. (Twice amended) The method of claim [26] 28, wherein subunits of said

[ASLV] AMV reverse transcriptase are expressed in said host cell to form said [ASLV] AMV reverse transcriptase.

117. (Twice amended) The method of claim 28, wherein said [one or more] subunits are encoded by nucleic acid sequences [of one or more ASLV reverse transcriptases] contained in one or more vectors.

118. (Twice amended) The method of claim 28, wherein [said subunits are] at least one subunit is [one or more] an α subunit[s].

119. (Twice amended) The method of claim 28, wherein [said subunits are] at least one subunit is [one or more] a β subunit[s].

120. (Twice amended) The method of claim 28, wherein [said subunits are] at least one subunit is [one or more] a βp4 subunit[s].

121. (Twice amended) The method of claim 28, wherein said subunits are an α subunit and a β subunit [of one or more ASLV reverse transcriptases].

122. (Once amended) The method of claim 119, wherein said β subunit[s] forms an [ASLV] AMV reverse transcriptase comprising two β subunits.

123. (Once amended) The method of claim 121, wherein said α and β subunits

form an [ASLV] AMV reverse transcriptase comprising an α and a β subunit.

127. (Three times amended) The method of claim 26, wherein said [ASLV] AMV reverse transcriptase has a polymerase specific activity from about 30,000 units per milligram to about 150,000 units per milligram.

128. (Twice amended) The method of claim 26, wherein said [ASLV] AMV reverse transcriptase has a polymerase specific activity from about 35,000 units per milligram to about 150,000 units per milligram.

129. (Twice amended) The method of claim 26, wherein said [ASLV] AMV reverse transcriptase has a polymerase specific activity from about 40,000 units per milligram to about 150,000 units per milligram.

130. (Twice amended) The method of claim 26, wherein said [ASLV] AMV reverse transcriptase has a polymerase specific activity from about 45,000 units per milligram to about 150,000 units per milligram.

131. (Twice amended) The method of claim 26, wherein said [ASLV] AMV reverse transcriptase has a polymerase specific activity from about 50,000 units per milligram to about 150,000 units per milligram.

132. (Twice amended) The method of claim 26, wherein said [ASLV] AMV

reverse transcriptase has a polymerase specific activity from about 55,000 units per milligram to about 150,000 units per milligram.

133. (Twice amended) The method of claim 26, wherein said [ASLV] AMV reverse transcriptase has a polymerase specific activity from about 60,000 units per milligram to about 150,000 units per milligram.

134. (Twice amended) The method of claim 26, wherein said [ASLV] AMV reverse transcriptase has a polymerase specific activity from about 65,000 units per milligram to about 150,000 units per milligram.

135. (Twice amended) The method of claim 26, wherein said [ASLV] AMV reverse transcriptase has a polymerase specific activity from about 70,000 units per milligram to about 150,000 units per milligram.

136. (Once amended) The method of claim 26, wherein said [ASLV] AMV reverse transcriptase has a polymerase specific activity from about 75,000 units per milligram to about 150,000 units per milligram.

137. (Once amended) The method of claim 26, wherein said [ASLV] AMV reverse transcriptase has a polymerase specific activity from about 80,000 units per milligram to about 150,000 units per milligram.

138. (Once amended) The method of claim 26, wherein said [ASLV] AMV reverse transcriptase has a polymerase specific activity of at least about 35,000 units per milligram.

139. (Once amended) The method of claim 26, wherein said [ASLV] AMV reverse transcriptase has a polymerase specific activity of at least about 40,000 units per milligram.

140. (Once amended) The method of claim 26, wherein said [ASLV] AMV reverse transcriptase has a polymerase specific activity of at least about 45,000 units per milligram.

141. (Once amended) The method of claim 26, wherein said [ASLV] AMV reverse transcriptase has a polymerase specific activity of at least about 50,000 units per milligram.

142. (Once amended) The method of claim 26, wherein said [ASLV] AMV reverse transcriptase has a polymerase specific activity of at least about 55,000 units per milligram.

143. (Once amended) The method of claim 26, wherein said [ASLV] AMV reverse transcriptase has a polymerase specific activity of at least about 60,000 units per milligram.

144. (Once amended) The method of claim 26, wherein said [ASLV] AMV reverse transcriptase has a polymerase specific activity of at least about 65,000 units per milligram.

145. (Once amended) The method of claim 26, wherein said [ASLV] AMV reverse transcriptase has a polymerase specific activity of at least about 70,000 units per milligram.

146. (Once amended) The method of claim 26, wherein said [ASLV] AMV reverse transcriptase has a polymerase specific activity of at least about 75,000 units per milligram.

147. (Once amended) The method of claim 26, wherein said [ASLV] AMV reverse transcriptase has a polymerase specific activity of at least about 80,000 units per milligram.

148. (Once amended) The method of claim 26, wherein said [ASLV] AMV reverse transcriptase comprises [one or more subunits] at least one subunit selected from the group consisting of [one or more] an α subunit[s], [one or more] a β subunit[s], and [one or more] a βp4 subunit[s,] of [one or more ASLV] AMV reverse transcriptase[s].

ABSTRACT

The invention relates to compositions comprising mixtures of polypeptides having reverse transcriptase (RT) activity and to methods of producing, amplifying or sequencing nucleic acid molecules using these compositions or polypeptides, particularly at temperatures above about 55°C. The invention also relates to nucleic acid molecules produced by these methods, to vectors and host cells comprising these nucleic acid molecules, and use of such nucleic acid molecules to produce desired polypeptide. The invention also relates to methods for producing Avian Sarcoma-Leukosis Virus (ASLV) RT subunits, in particular, Avian Myeloblastosis Virus (AMV) RTs, to isolated nucleic acid molecules encoding ASLV RT subunits, and to ASLV RT subunits produced by these methods. The invention further relates to nucleic acid molecules encoding recombinant RT holoenzymes, particularly ASLV RTs, methods for producing these RTs and to RTs produced by these methods. The invention also relates to kits comprising the compositions, polypeptides, and ASLV RTs of the invention.